SIGNIFICANCE TO NEW ZEALAND FORESTRY OF CONTAMINANTS ON THE EXTERNAL SURFACES OF SHIPPING CONTAINERS

P. D. GADGIL, L. S. BULMAN, R. CRABTREE

New Zealand Forest Research Institute,
Private Bag 3020, Rotorua, New Zealand

R. N. WATSON

AgResearch, Ruakura Agricultural Research Centre,
Private Bag, Hamilton, New Zealand

J. C. O'NEIL, and K. L. GLASSEY

Ministry of Agriculture and Forestry,
C.P.O. Box 39, Auckland, and P.O. Box 2526, Wellington, New Zealand

(Received for publication 27 October 1999; revision 7 July 2000)

ABSTRACT

A sample, comprising 3681 shipping containers, was selected randomly from containers landed at the ports of Auckland, Wellington, and Lyttelton in the period from September 1997 to May 1998. Each selected container was placed on a frame and all six sides of the container were examined for the presence of soil, plant, animal, or inorganic matter. Isolations for fungi were made from all soil samples found on containers (1150 in total) and nematodes (and other soil meso- and micro-fauna) were extracted from 347 soil samples. All plant material was examined microscopically for the presence of pathogens. The insects, spiders, and other animals were identified as far as possible and their pest status was determined. A container was classified as "quarantinable" if any of the contaminants found on it included either viable pests or viable fungi belonging to genera which include plant pathogens or plant parasitic nematodes. Of the 3681 containers examined, 2240 (61%) carried no contaminants, 580 (16%) carried non-quarantinable contaminants, and 861 (23%) carried quarantinable contaminants. Among the quarantinable contaminants were pathogenic species of *Fusarium* and a live egg mass of the gypsy moth *(Lymantria dispar* (Linnaeus)). The quarantinable contamination rate of containers originating in different parts of the world varied from region to region: for example, it was 13.7% for containers originating from Korea, Taiwan, and Japan; 20.9% for Northern Europe; 21.2% for North America; 28.3% for Australia; 33.2% for South-east Asia; 47.5% for the Pacific Islands; and 50% for South Africa. There were no regional differences in the proportion of quarantinable contaminants to the total number of contaminants and no differences in the quarantinable contamination rate were found for different cargo types or for different container types. It is concluded that the nature and the frequency of occurrence of contaminants on the external surfaces of shipping containers represent a risk to forestry in New Zealand. Further work needs to be carried out to quantify the magnitude of this risk.

New Zealand Journal of Forestry Science 30(3): 341–358 (2000)

Keywords: shipping containers; quarantinable contaminants; fungi; insects; soil; plant material.

INTRODUCTION

Generally, quarantine inspection of shipping containers is confined to the interior of the containers, and studies have been carried out on the risk to New Zealand forestry associated with cargo types, packing material, and country of origin of the contents (Bulman 1992, 1998). The external surfaces of containers, except for those suspected of carrying the giant African snail, have received little attention. In 1996, some containers from the Russian far east were found to be carrying egg masses of the gypsy moth *(Lymantria dispar)* on the outside. This brought home the point that no information was available on the potential of this particular pathway—the external surfaces of shipping containers—to introduce pests and pathogens which could damage New Zealand's forests. A study was initiated by the Ministry of Forestry to fill this gap. The aim of the study was to examine a sufficiently large sample of the containers landed in New Zealand over a period of several months to obtain a reliable idea of the nature of contaminants carried on the containers and their *potential* to serve as carriers of forestry pests and pathogens. The emphasis was therefore on determining whether a container harboured living organisms belonging to taxa with plant pathogenic, plant parasitic, or phytophagous species when it reached New Zealand. This paper reports the results of the study.

MATERIAL AND METHODS

Sample Size

Assuming (a) that contaminated containers are distributed randomly through the container population, (b) that the contamination rate is about 8% as shown in a small pilot study (A.Flux, pers. comm.), and (c) that the mean contamination rate should be established to within 10% (as assessed using a 95% confidence interval), a sample size of 5000 containers was initially determined upon following the binomial confidence limits tables of Mainland *et al.* (1956). As the study progressed, it became clear that the contamination rate was much higher than the assumed 8% and the sample size was reduced accordingly.

Sampling Method

Approximately 360 000 containers are landed annually at New Zealand ports. Most of these (89%) are landed at the three major ports of Auckland, Wellington, and Lyttelton and sampling of containers was limited to these ports. Based on the number of containers expected to be landed, each port was assigned a target number of containers to be selected for examination. The overseas ports at which the containers examined were loaded were classified according to either country or region (Table 1) and a target number of containers to be examined nationally from different countries and regions of the world was assigned later when an estimate of the contamination rate of containers originating from these regions could be made. One out of every 30 containers landed was initially selected for examination. The basic construction of all types of shipping containers is very similar, and size, although it will affect the amount of contaminants carried, is not likely to influence the type of the contaminants. Type and size were not, therefore, expected to affect the efficiency of

TABLE 1–Numbers of containers examined from different countries and regions

Country/Region	Major countries represented	No. of containers
Australia	Australia	669 (18.2%)
East Asia	Korea, Taiwan	227 (6.2%)
Far East	China, Eastern Russia	44 (1.2%)
Hong Kong	Hong Kong	479 (13.0%)
Japan	Japan	439 (11.9%)
North America	Canada, USA	415 (11.3%)
North Europe	UK, Belgium, France, Germany, Netherlands, Sweden	345 (9.4%)
Pacific Islands	Fiji, Samoa, Tahiti, Papua New Guinea, New Caledonia, Tonga	141 (3.8%)
Singapore	Singapore	358 (9.7%)
South Africa	South Africa	32 (0.9%)
South America	Chile, Argentina, Brazil	24 (0.7%)
South Asia	India, Pakistan, Sri Lanka	79 (2.1%)
South Europe	Spain, Portugal, Italy, Greece, Turkey	164 (4.4%)
South-east Asia	Malaysia, Thailand, Indonesia, Phillipines	196 (5.3%)
West Asia	Saudi Arabia, United Arab Emirates	69 (1.9%)
Total		**3681** (100%)

examination and no distinction was made by either category when selecting containers for examination. When the target number of containers to be examined from a given region was reached, no further containers originating from that region were included in the sample to be examined. Later in the study it became apparent that the target number of containers from some regions would not be reached at the current rate of selection and in March 1998 the selection rate for those regions was increased to 1 in 20.

Sampling Period

A study of the variability of the trade pattern showed that there was no significant monthly variation in the total amount of containerised cargo landed in New Zealand (data obtained from Statistics New Zealand). Analysis by country and by cargo group showed that imports from three countries in East Asia and the Far East showed significant monthly variation for some cargo groups. Imports from these countries in September, October, November, and December were significantly ($p<0.05$) higher. These months were included in the sampling period which ran from September 1997 to May 1998.

Container Examination Procedure

Examination of the external surfaces of containers was carried out by the Ministry of Forestry port quarantine officers.

Containers were randomly selected for examination from the ship's manifest before unloading began. The manifest was opened at any page and a container chosen at random. Every thirtieth container (every twentieth after March 1998 for selected regions) after this initial choice was then selected for examination. If a ship landed less than 30 containers, one

container was chosen at random. The selected container was placed on a stout frame so that the bottom could be safely examined. A powerful torch was used to aid examination of the twist-lock cavities and the space behind the bottom ledges. A ladder gave access to the sides and the top. Any soil or mineral contaminants (except stone chips) as well as all organisms and organic matter found were collected and placed in polythene bags which were then sealed and forwarded to the New Zealand Forest Research Institute for examination. Details of port of loading, region or country of origin of contents (this was not always available), container identifying marks, container type, size, and contents, and type and locations of contaminants found were entered for each container on a special form.

Contaminants

Soil

(a) Fungi. Soil samples from each container were thoroughly mixed and air-dried. Isolations for fungi were made from all samples received by sprinkling isolation plates with 0.1g of the air-dried sample. For the isolations three specialised media—Cycloheximide agar (Brasier 1981), a modified Nash-Snyder medium (Nelson *et al.* 1983), and PAR medium (Kannwischer & Mitchell 1978)—and a general medium (3% malt extract agar) were used.

Plates were incubated at 18°C. After 2 weeks, the plates were examined under a stereomicroscope and fruiting structures picked off and examined under a research microscope. Subcultures were made where further identification was desirable. Fungi were identified, as far as practically possible, to genus. Forty-four isolates of *Fusarium* from containers originating in different parts of the world were identified to species.

(b) Nematodes. Extraction of nematodes and other soil fauna began late in the study and altogether 347 samples were examined. These samples were not air-dried. The whole of each sample was set up in Whitehead trays (Whitehead & Hemming 1965) for nematode extraction. For the first batch of 41 samples, the sample elutriate was examined after the standard 48-h extraction period and re-examined after a further 5 days. Later batches were run for a 7-day extraction period to maximise nematode recovery. After extraction, the elutriate was placed in a 1-litre beaker and subsequently decanted into a smaller beaker after settling, leaving a 10-ml sample containing the nematodes and other soil fauna. This sample was transferred to a Doncaster dish and examined under a stereomicroscope. All micro- and meso-fauna were counted. Nematodes were assigned to the major feeding classes (bacterial, fungal, or plant-feeding) and plant parasitic nematodes were identified to genus.

Plant material

All plant material was examined under a stereomicroscope and fungal fruiting structures were picked off for closer examination under a research microscope. The incidence of fungi belonging to genera containing plant pathogens was noted.

Animal material

All insects, spiders and other invertebrates and the occasional vertebrate found were examined, under a stereomicroscope where necessary, and identified to order and in some cases to genus.

Classification of Contaminants

All contaminants found on the external surfaces of containers were classified as "potentially quarantinable" or "non-quarantinable" according to the threat they posed to the health of forest plants or forest products. Potentially quarantinable contaminants were:

(a) Soil samples which yielded either fungi belonging to genera which include forest plant or forest products pathogens (Holliday 1989), (for example, *Fusarium, Leptographium, Ophiostoma, Verticillium, Cladosporium*), or plant parasitic nematodes, or both;

(b) Woody or herbaceous plant material which carried viable fruiting structures which belonged to fungal genera which include pathogens (for example, *Phoma, Lophodermium, Cyclaneusma*);

(c) Live insect pests and viable egg masses of insect pests.

The term "quarantinable" applied to a contaminant customarily implies the presence of a "quarantine pest" which is likely to be deposited in a place suitable for its establishment and further spread, eventually leading to damage. We have assumed that if a potential quarantine pest is present in a mass of soil or as sporulating fruiting bodies on a plant part or as viable egg masses, then there is a reasonable chance that it could become established.

We also decided that no value was to be gained from attempting to establish whether any of the "potentially quarantinable" organisms were classifiable as "quarantine pests". The definition of a quarantine pest is "A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled" (FAO 1996). In the first place, the relevance to forestry of a list of quarantine pests is very questionable, given our very imperfect knowledge of forestry pests and pathogens and the great difficulty in predicting how an exotic organism would behave under New Zealand conditions. Secondly, it is very difficult, if not altogether impossible, to establish the pest status of fungal species. We classified all fungi which belonged to genera containing plant pathogenic species as potentially quarantinable, regardless of whether representatives of these genera or species were recorded as being present in New Zealand. The record of the presence of a morphologically-characterised taxonomic species in a country does not mean that all subspecies, formae speciales, varieties, or races of that species are present in the country. Most plant pathogenic fungal species are not a single genetic entity but contain many different forms which vary in pathogenicity and host specificity. These forms are morphologically indistinguishable. *Fusarium oxysporum* Schlechtendal provides a good example of such a variable species. Fifty-four formae speciales of *F. oxysporum*, many subdivided into a number of races, were recognised in the world in 1989 (Holliday 1989); only 12 of these were recorded in New Zealand (Pennycook 1989) and it would be impossible to determine from a morphological examination whether a new isolate of *F. oxysporum* (say, from soil adhering to a container) belonged to a forma specialis, let alone to a race, already present in New Zealand. To give another example, *Fusarium subglutinans* (Wollenweber & Reinking) Nelson *et al.* is recorded from maize in New Zealand (as *F. moniliforme* Sheldon var. *subglutinans* Wollenweber & Reinking (Pennycook 1989)); *F. subglutinans* f.sp. *pini* Correll *et al.* causes a very serious disease of *Pinus* spp. in North America (Correll *et al.* 1991) but it cannot be distinguished from the *F. subglutinans* on maize by morphological characters. It would be disastrous for New Zealand plantation forestry if all forms of *F. subglutinans* were allowed entry simply because one form is known to be

present here. To add to the difficulty, not all the forms within a species are recorded in the literature or even recognised. For example, no forms or varities of *Cyclaneusma minus* (Butin) DiCosmo *et al.*, a needle pathogen of *Pinus* spp. of worldwide distribution, are recorded in the literature but at least four forms of this fungus are present in New Zealand (P.D.Gadgil & A.Somerville, unpubl. data) and it is probable that there are many others in different parts of the world. These difficulties are generally recognised by quarantine authorities, most of whom regard all exotic pathogenic fungi as quarantinable. The Australian Quarantine and Inspection Service does not allow importation of *Pinus radiata* D.Don material which could carry *Dothistroma pini* Hulbary although this pathogen has been present in Australia since 1975. The United States Animal and Plant Health Inspection Service has prohibited the importation of *P. radiata* logs with *Diplodia pinea* (Desmazieres) Kickx infection, yet both the host and the pathogen are indigenous to the United States. We prefer to follow this sensible approach and have regarded all pathogenic fungi as undesirable immigrants which should be excluded as far as is practically possible. The same argument applies to plant parasitic nematodes.

In our view, all contaminants which we have classified as potentially quarantinable should be regarded as quarantinable for practical purposes. For example, if soil on a container originating from North America is shown to be carrying a species of *Fusarium*, then it is obviously capable of providing a pathway by which the highly pathogenic *Fusarium subglutinans* f.sp. *pini* could reach New Zealand, although this particular species may not be present in the particular soil sample examined. Soil, *per se*, should therefore be regarded as quarantinable. Following this logic, we have referred to all potentially quarantinable contaminants as quarantinable in the sections that follow.

RESULTS

The total number of containers examined was 3681 on which 3960 individual contaminants were found. Overall, 23.4% of the containers examined carried quarantinable (as defined in the section above) contaminants. The type and numbers of contaminants found are listed in Appendix 1. It will be appreciated that as a container frequently carried more than one contaminant, the number of contaminants listed in Appendix 1 is much greater than the number of contaminated containers. A container which carried one quarantinable contaminant was classified as quarantinable, although it may have also carried other non-quarantinable contaminants.

Soil Samples

Soil samples were collected from 1150 containers (as explained before, all soil samples from one container were combined) and fungal isolations were made from all samples. There were 722 containers contaminated with a low amount (10–50 g) of soil, 331 with a medium amount (50–500 g), and 97 with a large amount (>500 g) of soil. Out of these, 197 (17%) yielded only saprophytic fungi and 953 (83%) yielded fungi belonging to genera which include pathogens. Fungi belonging to the genus *Fusarium* were the most commonly isolated plant pathogens (633 soil samples yielded *Fusarium* spp.) and as some members of this genus are important forest pathogens, an attempt was made at identifying the number of species involved. *Fusarium* is a very large and complex genus and the identification of species within

it is a rather involved and time-consuming business. It was therefore necessary strictly to limit the number of isolates to be identified. Isolates were first grouped according to their appearance in culture on 3% malt agar slopes and representatives from culturally similar-looking groups were then selected for identification. This selection was influenced by the desirability of including isolates from as many regions as possible and, additionally, by the need to look closely at the isolates from North America because of the presence of the pine pitch canker pathogen (*F. subglutinans* f. sp. *pini*) in that region. Forty-four isolates were selected and identified to species (Table 2). Seven species were identified, with *F. oxysporum* as the most commonly encountered species. It must be borne in mind that this represents the minimum number of species present as isolates which appear similar in culture on 3% malt do not necessarily belong to the same species.

Altogether 347 soil samples were examined for the presence of nematodes and other soil animals. Of these 66 (19%) yielded no nematodes and out of the 279 samples which contained nematodes, only 15 (4% of the total) samples were classified as quarantinable because they contained plant parasitic nematodes.

TABLE 2–Species of *Fusarium* isolated from soil samples collected on containers originating from different countries and regions

Region/ Country	Total No. of isolates	Species of *Fusarium*	No. of isolates	Port of loading
Australia	143	*Fusarium oxysporum*	1	Brisbane
		F. equiseti	1	Melbourne
East Asia	18	*F. oxysporum*	1	Pusan
Far East	6	–	–	–
Hong Kong	55	*F. oxysporum*	1	Hong Kong
Japan	39	–	–	–
North America	64	*F. avenaceum*	1	Los Angeles
		F. culmorum	1	Los Angeles
		F. equiseti	3	Los Angeles
		F. oxysporum	15	Los Angeles, Seattle, Houston
		F. sambucinum	1	Los Angeles
		F. solani	4	Los Angeles
North Europe	50	*F. oxysporum*	2	Tilbury, Hamburg
		F. solani	1	Gothenberg
Pacific Islands	63	*F. solani*	1	Apia
Singapore	59	*F. oxysporum*	1	Singapore
South Africa	11	*F. equiseti*	1	Durban
		F. oxysporum	2	Durban
South America	6	–	–	–
South Asia	21	*F. longipes*	1	Karachi
		F. oxysporum	1	Calcutta
South Europe	34	*F. equiseti*	1	Lisbon
		F. oxysporum	1	La Spezia
South-east Asia	50	*F. oxysporum*	1	Manila
		F. solani	1	Port Kelang
West Asia	14	–	–	–
Total	**633**		**44**	

Types of Contaminants and their Location on the Containers

The types of contaminants found and their location on the containers are given in Table 3. As was expected, soil was the most frequent contaminant and most of it was found on the bottom of the container. Foliage and woody material were the next most frequently found contaminants. These were often quarantinable. Live insects and egg masses were found infrequently but some of them, such as the two egg masses (one of them viable) of *Lymantria dispar* (Asian gypsy moth), were of major quarantine significance. Other animals were rarely quarantinable and the indeterminate contaminants (bits of ropes, fishing lines, rags, bones, birds' nests, and such) had no forestry quarantine significance.

Contamination and Country or Region of Loading

The contamination rates of containers loaded on New Zealand-bound ships in differen regions are given in Table 4. The table also includes the binomial limits (95% confidenc intervals) from the tables of Mainland *et al.* (1956) for the percentages of containers carryin quarantinable contaminants. It is recognised that all contaminants were not necessaril acquired in the country or region of the port of loading but it (the port of loading) provide the only indicator to the origin of the contaminants. Containers originating from South Afric had the highest rate of quarantinable contamination (50.0%), followed closely by those fro ports of the Pacific Islands (47.5%). Containers from the Far East had the lowest quarantinabl contamination rate (13.6%). Containers from Japan and East Asia also had a low quarantinabl contamination rate (13.7%).

Although the quarantinable contamination rate of containers varied considerably betwee different regions, the percentage of quarantinable contaminants in the total number contaminants was very similar for all regions, ranging from 52.5% to 67.4% (Table 5).

The proportions of the types of contaminants—fungi, insects, or other—were also ve similar for the total number of contaminants found from all regions (Table 6).

The port of loading was used as the primary identifier for determining contamination rat because it is easily and accurately identifiable. Where possible, the country or region origin of the container contents was also recorded although this information is less readi available. Except for Hong Kong (44%) and Singapore (40%), more than 80% of t container contents originated in the region of the port of loading.

Contamination and the Contents of Containers

The types of goods inside the containers selected for examination were noted to whether there was any relationship between contamination rate and goods type. There v little difference in the quarantinable contamination rates for different types of goods, wh varied from 31.4% for stone and slate to 21.4% for machinery.

The percentage of quarantinable contaminants in the total number of contaminants fo did not vary widely, ranging from 79.5% for "unknown" contents to 54.1% for machine

The proportions of the types of contaminants were also very similar for the contamina found on containers of all goods types (fungi 38%, insects 7%, other 55%).

TABLE 3–Location and types of contaminants

Type	Location on container												Total	
	Bottom		Top		Door		Back		Rside		Lside			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Soil	1648	41.6	415	10.5	32	0.8	41	1.0	334	8.4	359	9.1	**2829**	**71.4**
Fruit/seed	38	1.0	28	0.7	2	0.1	1	0.0	2	0.1	2	0.1	**73**	**1.8**
Foliage	200	5.1	55	1.4	9	0.2	5	0.1	2	0.1	2	0.1	**273**	**6.9**
Woody material	166	4.2	119	3.0	5	0.1	2	0.1	3	0.1	6	0.2	**301**	**7.6**
Insects														
- Eggs	86	2.2	0	0.0	2	0.1	1	0.0	3	0.1	1	0.0	**93**	**2.3**
- Larvae/pupae	42	1.1	0	0.0	0	0.0	4	0.1	3	0.1	1	0.0	**50**	**1.3**
- Adults	46	1.2	12	0.3	0	0.0	6	0.2	5	0.1	0	0.0	**69**	**1.7**
Other animals	99	2.5	25	0.6	0	0.0	0	0.0	2	0.1	1	0.0	**127**	**3.2**
Indeterminate	109	2.8	26	0.7	1	0.0	2	0.1	2	0.1	5	0.1	**145**	**3.7**
Total	2434	61.5	680	17.2	51	1.3	62	1.6	356	9.0	377	9.5	**3960**	**100.0**

TABLE 4–Contamination rates for containers from different regions

Region of loading	No. of containers examined	No contaminants		Non-quarantinable contaminants		Quarantinable contaminants		95% conf. intervals
Australia	669	360	53.8%	120	17.9%	189	28.3%	24.7–31.5%
East Asia	227	163	71.8%	33	14.5%	31	13.7%	9.9–18.9%
Far East	44	33	75.0%	5	11.4%	6	13.6%	5.1–26.8%
Hong Kong	479	314	65.6%	74	15.4%	91	19.0%	15.6–22.7%
Japan	439	307	69.9%	72	16.4%	60	13.7%	10.7–17.8%
North America	415	277	66.7%	50	12.0%	88	21.2%	17.1–25.3%
North Europe	345	222	64.3%	51	14.8%	72	20.9%	16.5–26.0%
Pacific Islands	141	49	34.8%	25	17.7%	67	47.5%	38.5–55.6%
Singapore	358	217	60.6%	61	17.0%	80	22.3%	18.0–26.3%
South Africa	32	12	37.5%	4	12.5%	16	50.0%	31.9–68.1%
South America	24	12	50.0%	4	16.7%	8	33.3%	15.6–55.3%
South Asia	79	37	46.8%	17	21.5%	25	31.6%	22.0–43.4%
South Europe	164	99	60.4%	21	12.8%	44	26.8%	20.3–34.6%
South-east Asia	196	98	50.0%	33	16.8%	65	33.2%	26.5–39.9%
West Asia	69	40	58.0%	10	14.5%	19	27.5%	17.1–38.9%
Total	3681	2240	60.9%	580	15.8%	861	23.4%	21.7–24.3%

TABLE 5–Proportions of non-quarantinable and quarantinable contaminants from different regions

Region of loading	No. of contaminants found	Non-quarantinable contaminants		Quarantinable contaminants	
Australia	868	305	35.1%	563	64.9%
East Asia	140	65	46.4%	75	53.6%
Far East	40	19	47.5%	21	52.5%
Hong Kong	416	191	45.9%	225	54.1%
Japan	302	152	50.3%	150	49.7%
North America	364	121	33.2%	243	66.8%
North Europe	300	111	37.0%	189	63.0%
Pacific Islands	341	131	38.4%	210	61.6%
Singapore	389	162	41.6%	227	58.4%
South Africa	71	25	35.2%	46	64.8%
South America	43	14	32.6%	29	67.4%
South Asia	118	46	39.0%	72	61.0%
South Europe	177	62	35.0%	115	65.0%
South-east Asia	306	108	35.3%	198	64.7%
West Asia	85	34	40.0%	51	60.0%
Total	3960	1546		2414	

Contamination and the Types of Containers

The quarantinable contamination rates for the five different container types encountered in the course of the study are given in Table 7. The contamination rates for the 20-foot FCL containers and for reefers were very similar; the rate for the 40-foot FCL containers was a little higher as is to be expected from the greater length of these containers.